BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

The Patent Office

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP9 1RH

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

GB 99/00742

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Dated 25 March 1999

Patents Form 1/77

Patents Act 1977
(Rule 16)

THE PATENT OFFICE 12 MAR 1998 RECEIVED BY HAND

The Patent Office

D Y & CO

13MAR98 E345272-12 002246
P01/7700 25.00 - 9805301.0

Request for a grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. **Your reference** — P/4317.GB JAT

2. **Patent application number** *(The Patent Office will fill in this part)* — **9805301.0**

3. **Full name, address and postcode of the or of each applicant** *(underline all surnames)*

IMPERIAL COLLEGE OF SCIENCE,
TECHNOLOGY & MEDICINE
Sherfield Building
Exhibition Road
London SW7 2AZ

Patents ADP number *(if you know it)* 06704540 01

If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

4. **Title of the invention** — DETECTOR

5. **Name of your agent** *(if you have one)* — D YOUNG & CO

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

21 NEW FETTER LANE
LONDON
EC4A 1DA

Patents ADP number *(if you have one)* — 59006

6. **If you are declaring priority from one or more earlier patent applications, give the country and date of filing of the or each of these earlier applications and (if you know it) the or each application number**

Country	Priority application number *(if you know it)*	Date of filing *(day/month/year)*

7. **If this application is divided or otherwise derived from an earlier UK application, give the number and filing date of the earlier application**

Number of earlier application	Date of filing *(day/month/year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	NONE
Description	7
Claims(s)	0
Abstract	0
Drawing(s)	0

10. If you are also filing any of the following, state how many against each item.

Priority documents	NONE
Translations of priority documents	NONE
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	NONE
Request for preliminary examination and search *(Patents Form 9/77)*	NONE
Request for substantive examination *(Patents Form 10/77)*	NONE
Any other documents *(please specify)*	NONE

11. I/We request the grant of a patent on the basis of this application.

Signature

D Young & Co

D YOUNG & CO
Agents for the Applicants

Date

12 03 98

12. Name and daytime telephone number of the person to contact in the United Kingdom

J A TURNER 01703 634816

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 01645 500505

b) Write your answers in capital letters using black ink or you may type them

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you answered 'Yes' Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay please contact the Patent Office.

DETECTOR

Objectives

We will demonstrate an innovatory system for the sequencing of nucleic acids on a single semiconductor chip. This technology combines four key technologies already devised, developed and led by members of our collaboration. We fit squarely within biotech area 2.. *µDiaGene* demonstrates the synergistic power of our combining our individual technologies:

- intrinsic absorption imaging and sequencing of nucleic acids– an unconventional unlabeled approach -pioneered and demonstrated in the Imperial College (IC) physics dept.;
- the methods of analysis on a chip as pioneered and demonstrated at IC Centre for Analytical Sciences under in µTAS (Total Analysis Systems) programme;
- the development of UV-sensitised silicon pixel detectors, first applied in biomedical imaging and taken to its highest level of development at University of Cagliari (Cag) and
- the use of microridge sensor topologies (devised at IC) in Chemical Vapour Deposition Diamond, among whose leading proponents is our subcontractor the University of Lodz.
- Our team includes the foremost company involved in the manipulation of nanoliter quantities of biological materials, Evotec GmbH., who also provide a user-driven edge.

We will demonstrate a novel sequencing system based on the intrinsic absorption in nucleic acids of ultraviolet (UV) light. The demonstration step will comprise our producing nucleic acid sequences in four research laboratories, all well known as specialists for different sequences. Comparison at the single base-pair (Bp) level will be undertaken in which the same chip is operated both in the conventional mode using chemiluminescent labels and using our absorption technique. Naturally, the same sequence sample will also be checked on a conventional commercial system to provide extra checking redundancies. The *µDiaGene* system is based on the MIDIA-approach to molecular imaging for nucleic acid and protein sequencing and mapping, a technique developed at IC over the last three years and in the process of being commercialised. Its advantages are:

- the complete removal of mutagenic, toxic and carcinogenic chemi-, bioluminescent and radiolabels with associated benefits in running costs, health and safety, operational and training and disposal imperatives;
- an order of magnitude speeding of a typical sequence: a 6 hour (say) 500 Bp sequence electrophoresis can be achieved in 35 minutes in MIDIA and perhaps 10 minutes in *µDiaGene*. This saving in time comes because the image of bands identified by conventional labels is smeared by the isotropic emission of those labels (whether a radioactive label, or a laser-induced fluorescent detection (CE-LIF) system) while in the absorption technique we gain a factor of ten since the image of the nucleic acid band is the same size as the band (smeared slightly by diffraction). This can be made very small with µTAS technology. This is a new paradigm in sequencing.
- improved sensitivities and signal-to-noise in sub-microliter sequencing and imaging.

Our specific goal is to demonstrate these existing macroscopic technologies – developed in the last two years and in the process of being commercialised - at the chip level. *µDiaGene* can in principle also accept conventionally labelled nucleic acids to allow *in situ* cross checking. Our target sequences include: *Arabidopsis, Lactococus Lactis, HIV*, and an analysis of the spectrum of mutations in *Cystic Fibrosis, X-Linked Adrenal Hypoplasia*, and *Crigler-Lajjar Syndrome*.

The objective of this project is to demonstrate economic, advanced and automated high throughput megabase DNA sequencing technologies, based on novel nucleic acid detection systems, while minimising, perhaps to zero, any environmental or health and safety risks. Results are intrinsically digital, and given the exceptional sensitivities, rapid turn-around possible and high throughputs attainable, the *µDiaGene* technologies are ideally suited to the Human Genome research networks. Since the *µDiaGene* system allows pre-programmed sequence recognition masks to accompany any installation, in principle a "yes-no" answer could be obtained by relatively untrained end-users. A sequence triage system results, which coupled to the extremely low cost of this technology, can result in the wide dissemination of *µDiaGene* systems, to surgeries, schools and individual researchers' desk-tops.

2 Work Content

2.0 Introduction

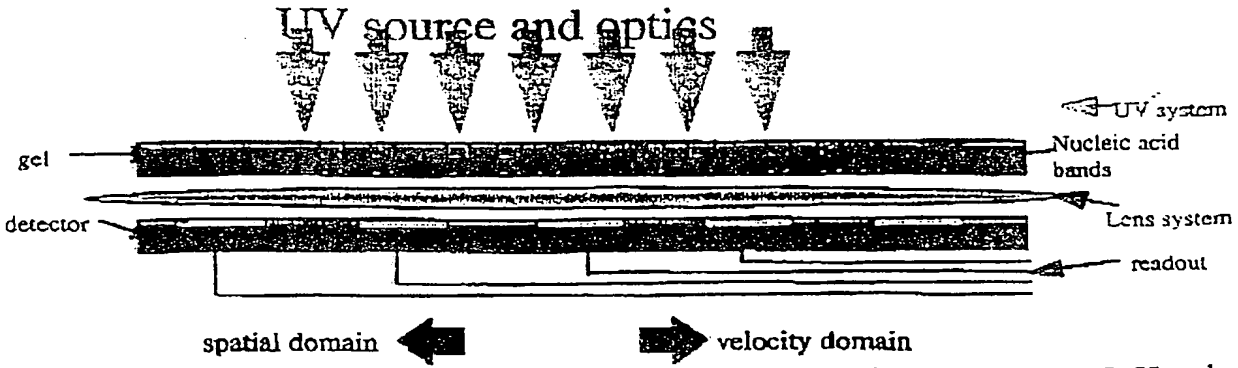
The current technologies use photographic techniques to image radioactive or luminescent tags attached to the nucleic acids - a time-consuming procedure, or phosphor imagers to record the sequences - an expensive alternative. Both processes use hazardous chemicals to tag the nucleic acids and the safe use and disposal of these is a major problem, and both require skilful scientific and technical input. ***Both are relatively slow. In any emission technique (eg. CE-LIF) the time-to-sequence (TTS) depends on the separation gradient (the electric field)and discriminator power (e.g. the capillary or electrophoresis gel) convolved with the size of the objects to be separated. The key ingredient in μDiaGene is the benefit miniaturisation brings. Reducing imaging dimensions by a factor 10 decreases time-to-sequence (TTS) by 100. By using intrinsic absorption, we reduce greatly the strong dependence on sample volume present in conventional emission techniques.***

μDiaGene will transform the results of four distinct research programmes into a working technology, suitable for commercialisation. We wish to emphasise that, while *μDiaGene* is definitely pre-competitive, our technology is such that the commercialisation process can be relatively fast. *μDiaGene* has substituted the technology of hardware, moving parts, chemistry and biological processes to one involving rapidly reproducible chip technology and software. *μDiaGene* will excel in an area which is a known to be a huge market, but improve upon existing technologies in every respect. The only area in which *μDiaGene* is at a nominal disadvantage compared with conventional technologies - handling nanoliters of biomaterials - is one in which two partners (IC and Evotec) have specialised and which they have solved. Our lean management structure and focused partnership has redundancy only where unforeseen circumstance could slow the rapid prototype development. The target: the *μDiaGene* sequencer chip - is well-defined.

2.1 The MIDIA programme.

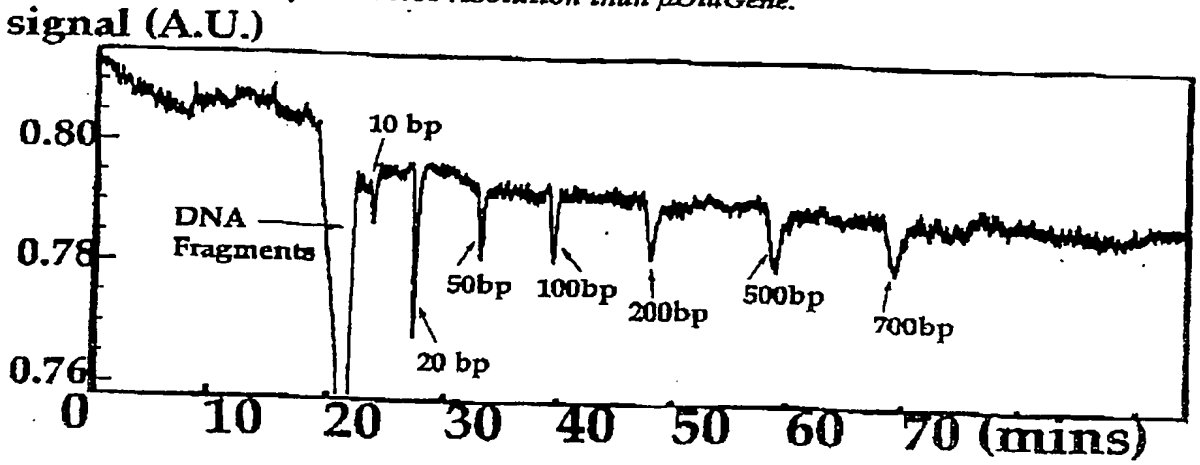
The MIDIA system has been shown to work using electrophoresis gels: polyacrylimide for sequencing and agarose for restriction enzyme maps. The technique is simple: DNA bands are separated in polyacrylimide gel, but are imaged not through radioactive emission or photon emission following UV laser activation (CE-LIF), but through the observation of a given band through intrinsic absorption: we make multiple images of the band shadows and build up a picture of their passage, sorting them into velocity (rather than spatial bands).

Figure 1: The side view of MIDIA technology.



The time-development of the separating bands of PCR marker can be seen in figure 2. Here bands have developed over time and as they progress along the polyacrylimide gel. By grouping signals in velocity space, we obtain the sequence. The figure shows a PCR ladder with bands corresponding to 10,20,50,100,200,500 and 700 base pairs with approximately 2ng/mL in each band. The plot shows how the MIDIA system sorts DNA clearly into a sequence. Note: Since taking this image, we have improved the signal to noise by about 100, of which a factor 50 is necessary to go to 1bp resolution where the bands are broadened, out at 700bp.

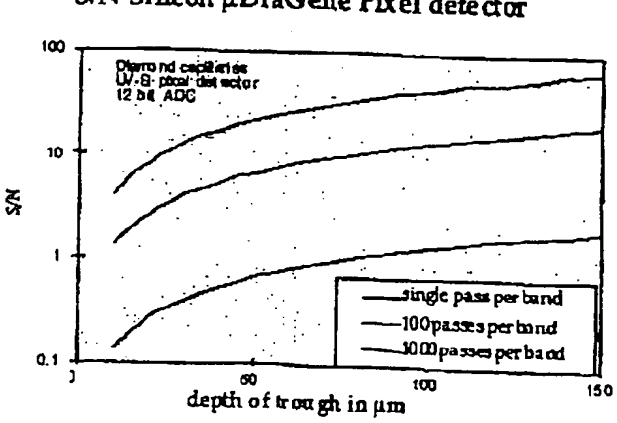
Figure 2: the PCR marker sequence after velocity-sorting: This data was taken in a system with at least a factor of 100 worse resolution than µDiaGene.



From these studies, we infer the *µDiaGene* system with 50 µm troughs will have a signal to noise over 1000 scans of better than 30:1 in separating adjacent base pairs, for sequences of more than 1 Megabp. Our sensitivities are clearly well within those used in most sequencing tasks and in any case, given our multiple measurements, can be increased arbitrarily. The TTS is simply a function of – and is approximately proportional to - the size of the objects being separated. In emission, DNA bands are 1-2mm. In MIDIA they are the size of the well, typically 500µm. In *µDiaGene* we propose to make DNA bands 50-100µm across. What would take 6 hours in a conventional system will take 35 minutes in MIDIA and 10 minutes in *µDiaGene*. The key issue is the signal to noise (S/N). Since we have measured all components, it is relatively straightforward and robust to combine them in a simulation. We obtain the following (figure 3) for 2ng/µL DNA, in 120µm wide microchannels:

As can be seen, a 50µm channel is perfectly adequate, since over a 18mm channel we will measure the DNA passage some 360 times. The limiting factor in S/N is, in fact, the photonic time-consistency. We therefore maintain a feedback system to stabilise of the UV source, and have a calibration channel to suppress all source-related noise. This has been proven in the MIDIA programme itself. With 150µm channels (as in *µDiaGene*) we have a big S/N redundancy.

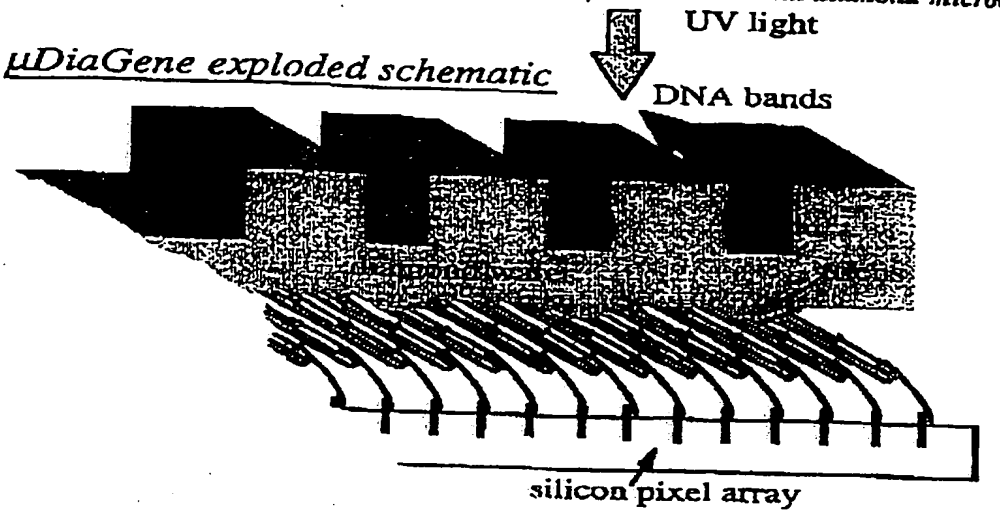
Figure 3:: The Signal to Noise in µDiaGene, as inferred from MIDIA data.



2.2 The *µDiaGene* programme

The objectives of *µDiaGene* are to demonstrate this technology on a diamond/silicon chip approximately 20 x 20 mm. A schematic is shown in figure 4 with the side view resembling figure 1. To prove this technology we will sequence a range of important biomolecules at our biotechnology laboratories in Cambridge, Imperial College Hamburg and Cagliari. This pan-Europe approach will allow a rapid and robust benchmarking and aid greatly in our ultimate technology dissemination.

Figure 4: The μDiaGene schematic , with silicon pixel detector and diamond microchannel plate.



2.3 *μDiaGene* Components

2.3.1 Light Source. The intrinsic absorption technique relies on the well-known fact that nucleic acids absorb strongly in the region around 257nm. Since this is close to the mercury emission line at 253.9nm, we have conventionally used a mercury lamp to illuminate and image the DNA bands.

2.3.2 Capillary microchannel plate. We have developed diamond microridge wafers for detector elements. In this application, we use unmetallised wafers as capillaries. The troughs are 20mm across; the depths can be 1-200 μm, and are etched by excimer laser by our subcontractor Exitech. The positions of the DNA and their velocities in the electrophoretic gel are precisely determined by measuring the photocurrent in the silicon pixel detector which is optically bonded to the diamond wafer. This technique works since diamond can be made to be one of the most transparent materials at 257nm. With a high refractive index (about 2.6 in this spectral region) we are able also to make microlenses on the diamond backplane to concentrate the light optimally on the silicon pixels. An additional complementarity is provided by the fact that an identical diamond/silicon detector system can be made to detect fluorescent emissions from conventional labels. Diamond has many advantages in this application, among them being:

- high breakdown voltage - diamond withstands $10^7 Vcm^{-1}$: our 20mm wafer must take 3KV;
- diamond is the best known thermal conductor, being 5 times better than copper;
- diamond is among the most transparent materials there is at 257nm;
- diamond has a very high refractive index: partial light concentration can be designed in;
- diamond is chemically inert and hydrophobic: it is completely biocompatible.

Figure 5 shows a scanning electron microscope image of a 20 μm ridged CVD diamond before laser cleaning. Two channels (the 5th and 6th from the right) have coalesced due to an error in the laser ablation process. This mistake was the only one found in this wafer's etching process. The image covers some 400 μm x 370μm.

Figure 5: a μchannel wafer in CVD diamond, with one manufacturing error.

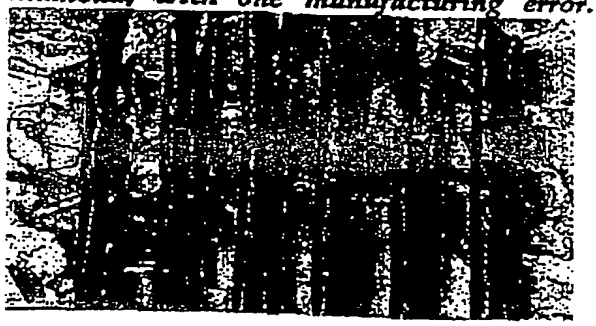


Figure 6: The channels are extremely cleanly etched: these are 20μ wide

2.3.3 *Silicon pixel detector* These have been developed to their highest level by Cagliari, who have used them in a range of biotechnological and medical applications. A silicon pixel device is better than a CCD here since it can be made much more cheaply in large quantities and does not suffer from the saturation a CCD could experience in this application. The pixels are made to match in one direction the size of the troughs - nominally 200 μm - and in the other dimension the size of the bands in the direction of their travel, which we take to be 150 μm. The pixels work equally well (with quantum efficiencies of over 90% and highly linear in response) in the UV as the X-ray region. The demonstration will also be made with UV-sensitised silicon photodiode arrays. However, this is for cross checks only: the pixel array is essential to get the channel density required on the wafer. The maximum density of sequencing lanes possible in the latter technology is 4 per mm; we intend to reach at least 200 channels in 20mm wide wafer.

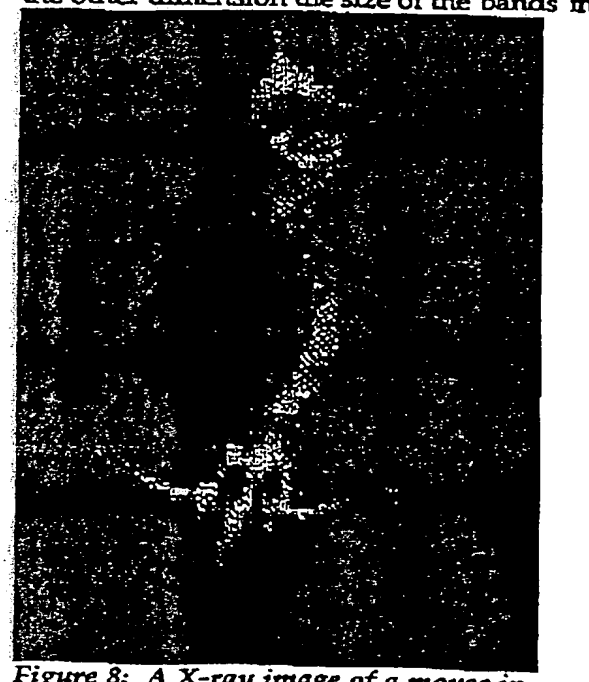
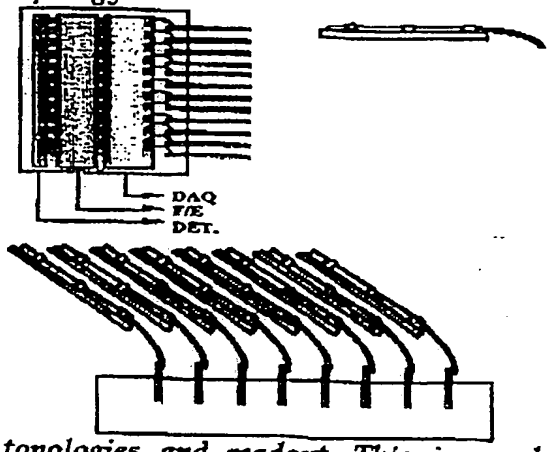


Figure 8: A X-ray image of a mouse in the Cagliari pixel detector with μDiaGene topologies and readout. This image has 170 x 170μm resolution and 16 bit intensity.

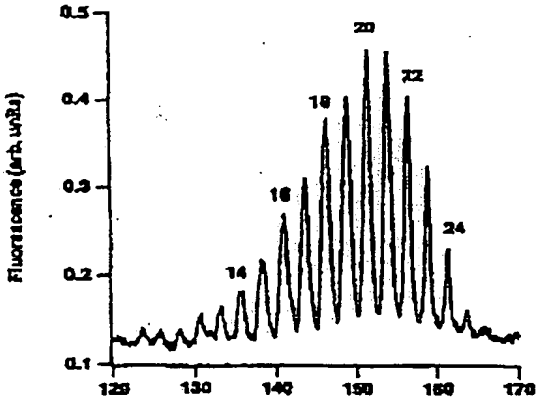
Figure 7 Pixel layout and interconnects in a Cagliari pixel detector with μDiaGene topology.



2.3.4 *μTotal Analysis System development:* The lead of Imperial College in the academic arena and Evotec GmbH's in the industrial arena in this area has over the last 5 years increased and been reinforced. The Manz group at IC will work closely with the Evotec GmbH team assigned to *μDiaGene* on all aspects of liquid handling and ancillary (non-optical, non-readout) instrumentation. The integration of the detection system and a multipurpose fluid interface to the macroworld onto the chip, has led to the integrated microsystem, with fluidic, optic and electronic components. We identify individual tasks as being: liquid handing and automation, mechanical micromanipulators, microdispenser syringe development, liquid electrostatic gating system and wafer re-use technology. The micromanipulation technological problems are demanding but well within the reach of

our collaboration. As is shown in figure 4, the microchannels which act as capillaries can h[illegible] just 150 μm deep, and 120μm wide and yet, as is shown in figure 3, achieve a S/N of 2[illegible] (2ng/μL DNA). The channels will be some 18mm long, in a 20 x 20 mm wafer. The liqu[illegible] handling is made easier by the ridges opening apertures: They are enlarged at their entrances to make a fan-in. This is possible since between the microridge "troughs" we have an extra 100 μm ridge top "deadspace". The demonstration system must therefore accept the nucleic acid mixture through a microsyringe system capable of delivering (say) 120 x 150 x 150 μm liquid = 1.5×10^{-6} l liquid into groups of five receptors 250μm across. Initially, we need only run only one group of 5 lanes (one for each termination and one calibration). The 18mm wide wafer can take some 3600 troughs in principle: we are not likely to want to attempt that in the Demonstration phase of this project, but any fluid handling system will be designed with the potential in mind:

Figure 9: μTAS sequencing



We will require :
multiple liquid input with 10% accuracies in quantity and 10 μm precisions in 3 dimensions. Evotec GmbH are experts in micro-separation methods, fluid handling etc. and have worked with IC for many years. The power of the μTAS approach is shown in the figure 9. This shows an electropherogram of a fluorescein-labeled phosphorothioate oligonucleotide mixture PS pd(T)10-25 mixture recorded at 1130 V/cm in a 10% T non-cross-linked polyacrylimide matrix, with chain lengths tentatively assigned, but clearly capable of 1bp separation. In Emission mode (as shown above), we sequence within a few minutes. It is possible we can reduce this in absorption mode.

2.3.5 *Readout and data acquisition:* we have designed and used a range of pixel readout systems.

2.3.6 *Analysis and Interpretation:* The pattern recognition will be adapted from the existing programs which have worked for macroscopic MIDIA. Note: *μDiaGene* output is intrinsically digital, and therefore can be collected and analysed remotely if necessary. However, we wish also to develop software masks, which can be supplied along with the hardware systems, which will allow inexpert use of the system and gain a first-pass assessment of a given sequence. Clearly, this approach will require extensive testing, and we have assigned a subcontractor, IDFA Cambridge, to coordinate this side of the exploitation.

2.3.7 *Demonstration and Benchmarking:* Our collaboration includes sequencing groups from Imperial College, Cagliari and Cambridge. IDFA Cambridge is a spin-off from University of Cambridge Department of Molecular Biology, and is part-originator of the MIDIA approach. They will decide upon and coordinate a concerted benchmarking and show-casing of the technology with cross-checks between commercial systems, *μDiaGene* in absorption mode and *μDiaGene* in emission mode. During this period, an important part of the coordination tasks will include liaison with potential EU partners who may wish to collaborate in commercialisation and exploitation of the new technology.

PCT/GB 99/04142

12-3-99

D young & Co.